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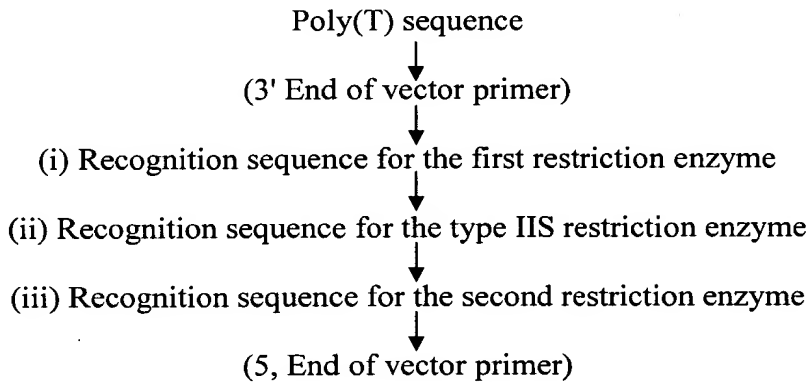
REMARKS

Claims 1-20 are pending in the present application.

The rejection of Claims 1-20 under 35 U.S.C. §112, first paragraph (enablement), is traversed.

Since it appears that the Examiner has not fully understood the present invention, Applicants provide the following summary. In amended Claim 1, the positions of the recognition sequences are re-defined by order from the poly(T) sequence without referring to the ends of the vector primer. As evidenced by Fig. 3E, the recognition sequences of the vector primer are arranged as follows:



Figs. 3 and 4 reveal that the portion between the 5' end and (iii) is removed in step G and the portion between 3' end and (i) is removed in step J. The lengths of the fragments removed are not important in the present invention. Therefore, it is sufficient to select the restriction enzymes and positions of their recognition sequences to satisfy the limitations (1) to (3) in amended Claim 1.

In regards to the Examiner's enablement rejection, we note that the Examiner has merely stated her conclusion that the claims contain inoperable embodiments. MPEP §2164.07 makes it clear that "when the examiner concludes that an application is describing an invention that is... inoperative... the burden is on the examiner to provide a reasonable basis to support this conclusion." Seemingly in support of the assertion of inoperativeness, the Examiner states that digestion of the vector primer as claimed in Claim 1 would give rise to the possibility that a vector primer lacking any cDNA insert is possible. However, this blanket assertion without any credible basis for that conclusion renders this assertion moot. Even if the Examiner were correct, MPEP §2164.08(b) states: "the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled." Accordingly, the Examiner has misapplied the inoperativeness standard even if the claims embrace inoperative embodiments.

Moreover, the Examiner has concluded that the figures and examples, which help guide the interpretation of the claimed invention, only provide a single interpretation of restriction site placement. However, the Examiner appears to have overlooked the important aspect provided by the claims, the figures, and the examples. Specifically, in the present invention, what is claimed and what is essential is the arrangement of restriction sites with respect to one another, as well as the order in which digestion occurs at each of these sites. The figures and examples ***do*** help guide the artisan as to how the claimed steps function and it is this guidance that permits the artisan to practice the claimed invention without undue experimentation (MPEP §2164.02).

As stated above, in amended Claim 1, limitations (1) to (3) have been added. In view of the limitations (1) to (3), one skilled in the art can easily select the restriction enzymes to be used and positions of their recognition sequences in the vector primer.

For example, the following combinations are mentioned in addition to the specific combination described in the present specification.

Combination	First restriction enzyme	Second restriction enzyme	Type IIS restriction enzyme	Figure
1. (Example in the present application)	*Pme*I	*Bgl*II	*Bsg*I	Figs. 1-5 in the present application
2	*Fse*I	*Bgl*II	*Gsu*I	Fig. 6A to Fig. 9N
3	*Not*I	*Bam*HI	*Mme*I	Fig. 10A to Fig. 13N
4	*Not*I	*Bgl*II	*Eco*57I	Fig. 14
5	*Pac*I	*Bgl*II	*Mme*I	Fig. 15
6	*Pac*I	*Bam*HI	*Mme*I	Fig. 16

* Figures 6-16 are **submitted herewith** to further illustrate the present invention (the numbering follows from originally presented Figures 1-5). The procedures shown in these figures illustrate combinations 2-6 above and demonstrate that every combination is practicable.

The above combinations can be theoretically derived from the disclosure of the present specification, in particular, in view of the limitations (1) to (3). Based on the foregoing, Applicants submit that the present claims are fully enabled by the specification and the common knowledge available in the art and, as such, Applicants submit that the skilled artisan may easily make many additional combinations in view thereof.

The rejection should be reconsidered and withdrawn.

The rejection of Claims 1-3 under 35 U.S.C. §103(a) over Kinzler et al in view of Okubo et al is obviated in part by amendment and traversed in part.

At the outset, Applicants would like to thank the Examiner for indicating that Claims 4-20 are free from the disclosure of Kinzler et al and Okubo et al.

Applicants note that the Examiner has maintained this ground of rejection "because of the lack of clarity in the claims" and the maintained indefiniteness rejection (see below). In view of the amendments and arguments set forth herein to obviate the Examiner's rejections of the claims as being indefinite and lacking enablement, Applicants believe that this ground of rejection is now moot. However, for sake of completeness, Applicants reassert the arguments from the Amendment and Request for Reconsideration filed on June 25, 2003 with additional comments thereafter.

In the rapidly exploding world of genomic research, a wealth of putative genes and proteins are being identified and indexed. Although the indexes (databases) are extremely helpful for determining the identity and/or activity of a putative gene and/or protein, these databases provide little if any direction with respect to the expression patterns and expression frequencies of the genes. To further confuse the issue, not all genes in a cell are transcribed into mRNA and ultimately translated into proteins. It is estimated that the human cell will express approximately 15,000 genes with the expression frequency of each gene responding to several environmental factors, such as cell type and external stimuli.

Current efforts have been focused on measuring the total types of mRNA; however, measurement of genetic expression frequency information based on total proteins has remained elusive. Heretofore, the most effective means for genetic expression profile analysis has been the method of serial analysis of gene expression (a.k.a. SAGE) as set forth by Kinzler et al (U.S. 5,695,937).

As set forth in Kinzler et al (see columns 3-8) and summarized in the present specification, in SAGE cDNA is produced by using a poly(T) having the 3' end bonded with biotin as a primer, the cDNA is digested with a restriction enzyme (a.k.a. an "anchoring enzyme"), the cDNA fragments containing the 3'-biotinare adsorbed on avidin beads, the

beads are divided into two portions, and two distinct linkers (A or B) are ligated to the cDNA fragments (about 13 bp) adsorbed on either portion of the divided bead population (see page 5, line 27 to page 6, line 8 of the present specification). Each linker (A or B) contains a site for a Class II restriction enzyme, termed a "tagging enzyme" (see page 6, lines 8-10 of the present specification). The cDNA fragments are then excised from the beads with the tagging enzyme, blunt-ended at the excised end, and the tags are ligated to the linker A and the liner B are connected (see page 6, lines 10-14 of the present specification). Accordingly, the product of this connection is termed a "ditag" (see page 6, lines 14-15 of the present specification). Subsequently, the ditag is amplified by PCR using primers that recognize linker A and linker B and a large number of amplified ditags are ligated, inserted into a vector, and sequenced (see page 6, lines 15-19 of the present specification). Accordingly in the SAGE method, 50 tag sequences can be determined by one round of sequencing from which the expression frequency may be calculated (see page 6, lines 19-22 of the present specification).

However, the SAGE method disclosed by Kinzler et al is saddled with many problems and/or deficiencies, as the method could not be reproduced in most research facilities (see page 7, lines 10-13 of the present specification). For example, the SAGE method is very complicated and requires specially trained people to administer. Moreover, for each measurement nearly 1 μg of mRNA is required, which is nearly impossible to obtain from clinical biopsies or micro tissue samples (see page 7, lines 16-22 of the present specification).

Further, the SAGE method is prone to errors due to its reliance on the accurate determination of the sequence of a very short (13 bp) tag (see page 7, lines 23 to page 8, line 5 of the present specification). A significant source of the errors affecting the applicability of the SAGE method arises from the fact that the SAGE method requires the formation of a

ditag resulting in a poorly defined border region. Specifically, due to the nature of the restriction enzymes selected, the tags that are interconnected to form the ditags inherently are of different lengths and become intermingled. Therefore, upon ligation it is extremely difficult to determine where one tag ends and the next one begins.

Another source of errors associated with the SAGE method is the use of an avidin/biotin capture method. The avidin/biotin capture method lends to an increase in contamination further increasing the requirement for an increased quantity of mRNA to obtain data.

As stated in the present specification at page 9, line 12 to page 10, line 2, in actual laboratory practice, the SAGE method possesses many drawbacks, which includes:

1) the techniques required for the method are complicated and they can be performed only by specially trained persons;
2) about 1 μg of mRNA is required for the measurement, and therefore it is substantially impossible to perform the measurement with a sample that can be obtained in a small amount, for example, a clinical biopsy material, and it is similarly impossible to measure difference of genetic expression in micro tissue portions; and
3) the method theoretically causes considerable measurement errors because a ditag is measured.

Okubo et al merely provide an arrangement of restriction sites with respect to the 3' poly(T) sequence, therefore Okubo et al does not compensate for the myriad of deficiencies in the disclosure of Kinzler et al

In order to solve these problems associate with the art of record, Applicants have surprisingly developed the following method for analyzing expression frequencies of genes called MAGE (Micro-Analysis of Gene Expression):

(a) forming a vector primer to which each cDNA is ligated, by annealing the vector primer with each mRNA derived from a cell of which expression frequencies of genes is to be analyzed, and synthesizing the cDNA, said vector primer comprising a linear plasmid

vector having a single-stranded poly(T) sequence at one 3' end of one strand of the linear plasmid vector, said linear plasmid vector comprising a recognition sequence for a first restriction enzyme, a recognition sequence for a type IIS restriction enzyme, and a recognition sequence for a second restriction enzyme in order from the poly(T) sequence, wherein (1) the first restriction enzyme and the second restriction enzyme each digest the vector primer at one position, (2) the cleavage site of the type IIS restriction enzyme is positioned beyond the recognition sequence of the second restriction enzyme, and (3) the vector primer digested with the first restriction enzyme and the type IIS restriction enzyme can be cyclized,

(b) digesting the vector primer to which the cDNA is ligated, with the second restriction enzyme and a third restriction enzyme that does not digest the vector primer and forms a digested end of the same shape as a digested end obtained with the second restriction enzyme, to excise an upstream region of the cDNA, and cyclizing the vector primer,

(c) digesting the cyclized vector primer with the first restriction enzyme and the type IIS restriction enzyme to excise a downstream region of the cDNA so that a tag consisting of a part of the cDNA is left, and cyclizing the vector primer again,

(d) performing polymerase chain reaction (PCR) by using the vector primer as a template and primers to amplify the tag, wherein said primers are oligonucleotides having nucleotide sequences corresponding to known nucleotide regions on each side of the tag that are maintained in the vector primer following digestion in step (c),

(e) ligating the amplification products to form a concatemer of the tags, wherein the tags are separated by known nucleotide sequences introduced by the primers for tag amplification ***so that no ditags are present in the concatemer***, and

(f) determining the nucleotide sequence of the concatemer and investigating types and frequencies of tags occurring in the nucleotide sequence (see Claim 1).

Applicants wish to draw the Examiner's attention to the fact that the claimed invention (above) is neither disclosed, nor suggested by the combined disclosures of Kinzler et al and Okubo et al. Specifically, Kinzler et al requires the formation of ditags (see Claims 1-43 and column 2, line 21 to column 14, line 22), whereas the present invention has specifically excluded the formation of ditags (see Claim 1, step (e)). Such a disclosure by Kinzler et al *teaches away* from the claimed invention. Furthermore, Okubo et al merely provide an arrangement of restriction sites with respect to the 3' poly(T) sequence and does not compensate for the teaching away from the present invention by Kinzler et al.

Moreover, the present invention, in which a ditag is not produced, solves the prior art problems of the indefinite border between tags. Since a sample can be amplified by PCR several times, the present invention permits the analysis of sub-micro quantities of mRNA (see page 27, line 9-12 of the present specification). In addition, since the present invention uses a vector primer for cDNA synthesis, the cDNA may be directly fused to the vector and, therefore, analysis can be performed without using an avidin/biotin capture system, thus avoiding the introduction of additional contaminants that plagued the SAGE method (see page 27, lines 12-16 of the present specification).

Citing In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974), MPEP §2143.03 states: "To establish a prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." Applicants submit that the combined disclosures of Kinzler et al and Okubo et al fail to meet this requirement, and as such the artisan would have no direction to practice the claimed method in which ditags are not formed in the concatemer, much less the advantageous properties flowing therefrom.

Accordingly, the present invention would not be obvious in view of the combined disclosures of Kinzler et al and Okubo et al.

Moreover, as stated above, the method of the present invention is primarily characterized by using the specific vector primer as defined in Claim 1. By this feature, a tag that is flanked by known sequences can be obtained (Fig. 4L), whereby a concatemer in which tags are ligated via a sequence that enables recognition of ends of the tags can be formed (see Fig. 5M and page 10, lines 18-20 of the present specification). The formation of the concatemer is not expected in view of the disclosures of Kinzler et al and Okubo et al, either individually or in combination.

Kinzler et al disclose a method for serial analysis of gene expression in which formation of a ditag is necessary, and a concatemer comprising the ditags is used (see Kinzler et al Fig. 1B). Kinzler et al never realize the problems associated with using a ditag. Specifically, Kinzler et al fails to recognize the problem in that the border between tags becomes indefinite (see page 27, lines 3-9 and page 7, line 5 from the bottom to page 8, line 19 of the present specification). In particular, the cleavage site of the type IIS restriction enzyme that is used as the tagging enzyme in Kinzler et al (col. 5, lines 53-62) is not always stable.

For the Examiner's convenience in understanding this disclosure, a copy of restriction enzyme catalog is attached. As seen from the notes regarding *Bsm*FI and *Hph*I, these cleavage sites are not stable. Therefore, in Fig. 1B of Kinzler et al, it is shown that 9-mer tags are excised by using *Fok*I, but lengths of actual obtained tags may vary between 8-mer to 10-mer. Accordingly, the lengths of the resultant ditags may vary between 16-mer to 20-mer. For example, when a 20-mer ditag is sequenced, there are many variations such as 10-mer +

10-mer, 9-mer + 11-mer, 8-mer + 12-mer and the like. The lack of boundary definition between tags causes considerable errors in investigating types and frequencies of tags.

In contrast, when each tag is flanked by known sequences as in the present invention, the tag can be definitively identified; whereby the errors associated with the prior art techniques are eliminated. Formation of the concatemer in which each tag is flanked by known sequences and the advantage thereof are not expected by Kinzler et al.

Okubo et al merely relates to a system for large-scale sequence of cDNA by PCR amplification, in which PCR reaction products are directly subjected to sequencing (abstract of Okubo et al). The restriction enzymes are selected to clone the 3' end fragment of cDNA (Figure 1 of Okubo et al). Okubo et al are silent about tagging and concatenation. Therefore, Okubo et al can not compensate for the deficiencies in the disclosure of Kinzler et al.

In the outstanding Office Action, the Examiner states that the improvements that distinguish the present invention from that of Kinzler et al is not recited in the claims. This assertion is clearly in error as (e) specifies that ***no ditags are present in the concatemer*** (see Claim 1). Therefore, the distinguishing feature is, *in fact*, present in the claims.

In view of the foregoing, Applicants respectfully request withdrawal of this ground of rejection.

The rejection of Claims 1-20 under 35 U.S.C. §112, second paragraph, is obviated by amendment.

A. The Examiner asserts that the phrase "near the 5' end" in Claim 1 to be an undefined relative term. Applicants have deleted this phrase from Claim 1. Therefore, this rejection is believed to be moot.

B. The Examiner asserts that the phrase "at a downstream position from the recognition sequence for the second restriction enzyme" in Claim 1 to be indefinite as lacking a standard for ascertaining the requisite degree. Applicants have deleted this phrase from Claim 1. Therefore, this rejection is believed to be moot.

C. The Examiner asserts that the phrase "to amplify the tag" is indefinite. In amended Claim l, it is clear that the type ITS restriction enzyme digests the upstream region of cDNA so that a part of cDNA is left on the vector primer in the step (c). As discussed above, from the description of the present specification, it is clear that the type IIS restriction enzyme and its position are selected so that the cleavage site of the type IIS restriction enzyme is positioned beyond the recognition sequence of the second restriction enzyme. In view of the limitation (2) in Claim 1, it is clear that the tag is always present after the step (c) (see Fig. 3H and Fig. 4I).

Moreover, the Examiner appears to have overlooked the phrase "to excise a downstream region of the cDNA so that a tag consisting of a part of the cDNA is left, and cyclizing the vector primer again" in step (c). As this limitation clearly indicates, the tag is defined as being the portion of the cDNA that is left following digestion with the type IIS restriction enzyme. Further, Applicants note that Figures 2-4 provide a clear roadmap for the Examiner to understand the claims

In view of the foregoing, withdrawal of this ground of rejection is requested.

The Examiner has acknowledged the claim to foreign priority under 35 U.S.C. §119(a)-(d) to JP 11-038538, filed February 17, 1999; however, the Examiner has again indicated that the claim to priority of PCT/JP00/00902 has not yet been granted. Apparently, the Examiner has not acknowledged priority because the foreign priority document has not

yet been received (see Office Action of September 26, 2003, page 2). The Examiner has indicated that she received a 109-page document, apparently from the International Bureau, on July 30, 2002 that corresponds to PCT/JP00/00092, not PCT/JP00/00902. Applicants note that this appears to be a problem between the Examiner and the International Bureau. Applicants again submit that the present application is a 371 of PCT/JP00/00902, filed on February 17, 2000, and note that the obligation to obtain the priority document from the International Bureau pursuant to PCT Rule 17.2(a) is upon the Office (see MPEP §1893.03(c)).

Applicants request that, in the next communication, the Office acknowledge their claim to priority and acknowledge that copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau.

Finally, the Examiner has made the present rejection final asserting the Applicants amendment necessitated the enablement rejection. Applicants disagree with this assertion by the Examiner noting that, for the reasons set forth herein, is improper and even if the rejection were proper it has not been created by the amendment filed on June 25, 2003. If the previously pending claims lack an enabling disclosure for the Examiner's asserted reasons, then Applicants note that this rejection should have been made in the first action on the merits. The fact that the Examiner failed to timely assert this rejection (i.e., piecemeal examination; MPEP 707.07(g)) should not be held against the Applicants. Accordingly, it is respectfully requested that the Examiner acknowledge the impropriety of the "final" status of the outstanding Office Action and that any ensuing rejection be in a new, non-final Office Action.

Applicants submit that the present application is now in condition for allowance.

Early notification of such action is earnestly solicited.

Respectfully submitted,

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NEW ENGLAND BioLabs Inc.

2002-03 Catalog & Technical Reference

BsmB I

NEBuffer 1 2 3 4
% Activity 75 100 100 100
NEB3 55° Yes

Source: *Bacillus stearothermophilus* B61 (Z. Chen)

Reaction Conditions: NEBuffer 3
100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C). **Incubate at 55°C.**

Ligation and Recutting: After 2-fold overdigestion with BsmB I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent B, see p. 237.

Heat Inactivation: 80°C for 20 minutes.

Note: BsmB I is an isoschizomer of Esp3 I.

Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 252).

Incubation at 37°C results in 20% activity.

#R0580S	200 units
#R0580L	1,000 units

5′. . . C G T C T C (N)$_1$▼. . . 3′
3′. . . G C A G A G (N)$_5$▲. . . 5′

BsmF I

NEBuffer 1 2 3 4
% Activity 25 50 50 100
NEB4 BSA 65° dam Yes

Source: *Bacillus stearothermophilus* F (Z. Chen)

Reaction Conditions: NEBuffer 4 + BSA
50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. **Incubate at 65°C.**

Ligation and Recutting: After 10-fold overdigestion with BsmF I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 2,000 units/ml.
Assayed on pBR322 DNA.

Storage Conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: 80°C for 20 minutes.

Note: BsmF I is an isoschizomer of Fin I. Occasionally, BsmF I has been shown to cleave the sequence GGGAC(9/13). The exact frequency of this occurrence has yet to be determined.

Blocked by overlapping *dam* methylation (see p. 253). Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 252).

Incubation at 37°C results in 50% activity.

#R0572S	100 units
#R0572L	500 units

5′. . . G G G A C (N)$_{10}$▼. . . 3′
3′. . . C C C T G (N)$_{14}$▲. . . 5′

BsoB I

NEBuffer 1 2 3 4
% Activity 10 100 100 50
RH NEB2 37° No

Source: An *E. coli* strain that carries the cloned BsoB I gene from *Bacillus stearothermophilus* JN2091 (D. Clark)

Reaction Conditions: NEBuffer 2
50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 40-fold overdigestion with BsoB I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

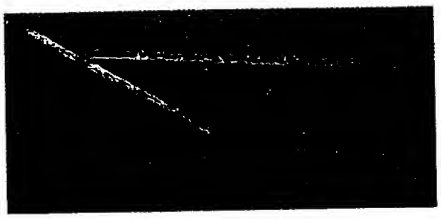
Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: No, see p. 240.

Note: BsoB I is a thermophilic isoschizomer of Ava I.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity (see p. 245).



BsoB I: DNA cocrystals (Mark van der Woerd and Alan Friedman, Purdue University; John Pelletier, Hong Ruan, Laurence Ettwiller and Shuang-yong Xu, New England Biolabs)

#R0586S	10,000 units
#R0586L	50,000 units

5′. . . C▼Py C G Pu G . . . 3′
3′. . . G Pu G C Py▲C . . . 5′

BSA Requires BSA dam Methylation Sensitivity Yes Heat Inactivation Blue/White Certified

HinP1 I

NEBuffer 1 2 3 4
% Activity 100 100 100 100

RR NEB2 37° Yes

Source: An *E. coli* strain that carries the cloned HinP1 I gene from *Haemophilus influenzae* P_1 (S. Shen)

Reaction Conditions: NEBuffer 2
50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 20-fold overdigestion with HinP1 I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: HinP1 I is an isoschizomer of Hha I. HinP1 I produces a 5′ extension, whereas Hha I produces a 3′ extension. The 5′ extension can be efficiently ligated into the Acc I site of M13 and pUC cloning vectors.

Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 252).

#R0124S	2,000 units
#R0124L	10,000 units

5′. . . G▼C G C . . . 3′
3′. . . C G C▲G . . . 5′

Hpa I

NEBuffer 1 2 3 4
% Activity 25 50 10 100

RR NEB4 37° No

Source: An *E. coli* strain that carries the cloned Hpa I gene from *Haemophilus parainfluenzae* (ATCC 49669)

Reaction Conditions: NEBuffer 4
50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 50-fold overdigestion with Hpa I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 5,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: No, see p. 240.

Note: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 252).

Conditions of high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity (see p. 245).

#R0105S	500 units
#R0105L	2,500 units

5′. . . G T T▼A A C . . . 3′
3′. . . C A A▲T T G . . . 5′

Hpa II

NEBuffer 1 2 3 4
% Activity 100 50 10 100

RR NEB1 37° Yes

Source: An *E. coli* strain that carries the cloned Hpa II gene from *Haemophilus parainfluenzae* (ATCC 49669)

Reaction Conditions: NEBuffer 1
10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.0 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 10-fold overdigestion with Hpa II, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 and 50,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: Hpa II is an isoschizomer of Msp I.

Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 252).

Inhibited by salt concentrations of > 50 mM KCl.

#R0171S	2,000 units
#R0171L	10,000 units

for high (5X) concentration, order #R0171M (10,000 units)

5′. . . C▼C G G . . . 3′
3′. . . G G C▲C . . . 5′

Hph I

NEBuffer 1 2 3 4
% Activity NR 75 0 100

RR NEB4 37° dam Yes

Source: An *E. coli* strain that carries the cloned Hph I gene from *Haemophilus parahaemolyticus* (ATCC 49700)

Reaction Conditions: NEBuffer 4
50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 3-fold overdigestion with Hph I, approximately 50% of the DNA fragments can be ligated. Of these, > 95% can be recut.

Concentration: 5,000 units/ml.
Assayed on λ DNA.

Storage Conditions: 150 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at –20°C.

Diluent Compatibility: Diluent B, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: Hph I may cleave at N_9/N_8 depending on the sequence between the recognition and cleavage sites (Cho, S.-H. and Kang, C. (1990) *Mol. Cells* 1, 81–86). Incubation of > 12 units for over 4 hours on φX174 DNA results in additional cleavage products. This has not yet been shown to occur on other DNAs. Low pH and high glycerol concentration enhance this activity.

Blocked by overlapping *dam* methylation (see p. 253).

#R0158S	400 units
#R0158L	2,000 units

5′. . . G G T G A $(N)_8$▼. . . 3′
3′. . . C C A C T $(N)_{7}$▲. . . 5′

BSA Requires BSA dam Methylation Sensitivity Yes Heat Inactivation Blue/White Certified